Report of the land-based testing

by

MICROFADE BWMS

March 25, 2011

Laboratory of Aquatic Science Consultant Co., Ltd.

Statement

Test Title

Land-based testing by MICROFADE BWMS

Test Number

K-08-2

The test was implemented on the basis of the quality management plan (QMP) to which the technical requirements of ISO/IEC 17025 are added while using the quality management system established, implemented, and maintained in conformity to ISO 9001:2000 to consistently provide products that satisfy the customer requirements of the Laboratory of Aquatic Science Consultant Co., Ltd., and applicable regulation requirements, as well as to achieve improvements in customer satisfaction by all parties concerned through continuous improvements to the system and effective application thereof. The test was implemented according to the quality management system established, implemented, and maintained, and in which the technical requirements are stipulated, according to ISO 9001 (2010) of the Laboratory of Aquatic Science Consultant Co., Ltd.

For the test, the quality assurance and accreditation project (QAAP) that includes implementation of the test methods, such as the analysis method of measurement items according to the criteria described below was prepared, and the test was implemented as per the project.

Responsible Party for the Test:

Laboratory of Aquatic Science Consultant Co., Ltd.

(March 25, 2011)

(Kushiro Kei)

Reliability Assurance Statement

Test Title

Land-based testing by MICROFADE BWMS

Test Number

K-08-2

Regarding the test, inspections or audits by the party responsible for reliability assurance work were implemented as per the table below, and the results thereof were reported to the responsible person for the test.

Implementation Date of Inspection/Audit	Type of Inspection and Audit/Inspection Item	Date of Report
November 20, 2009 March 10, 2010 (1st Edit) March 5, 2011 (Final Edit)	Test Plan	November 30, 2009 March 19, 2010 (1st Edit) March 10, 2011 (Final Edit)
January 12, 2010	Test Facility/Equipment, apparatuses, SOP, etc.	January 13, 2010
January 21, 2010 and January 26, 2010	Implementation State of Test	January 27, 2010
December 10, 2010	Report (draft)	December 13, 2010
March 19, 2011	Final Report	March 25, 2011

The undersigned assures that the above-stated tests were implemented according to the Test Plan, and the raw data obtained in the tests concerned were accurately reflected in the statement of the Report.

Responsible Party for Reliability Assurance Work:
Laboratory of Aquatic Science Consultant Co., Ltd.

大木康弘 (March 25, 2011)

(Ooki yasuhiro)

THE TESTING PROCESS OF LAND-BASED TESTING

1. Application

The land-based testing by MICROFADE BWMS (Model: MF-250) is performed based on the "Guideline for approval of ballast water management systems (G8)" specified on Resolution MEPC.174 (58).

2. Test facility

Marine Technology Institute Corporation
2269-53 Seto-cho, Imari-city, Saga-pref., 848-0043, JAPAN
TEL +81-955-23-2266

3. Test organization

Laboratory of Aquatic Science Consultant Co., Ltd.
1-14-1, Kamiikedai, Ohta-ku, Tokyo, 145-0064, JAPAN
+81-3-3748-5900

4. Test schedule

Two sets of test cycles were conducted, one was seawater (>32 PSU) and the other was brackish water (<22 PSU). Each test was repeated five times.

Table 1 Test Cycles

Test cycles		Date of ballasting	Date of deballasting
Brackish water	Run 1	January 21, 2010	January 26, 2010
	Run 2	January 31, 2010	February 5, 2010
	Run 3	February 10, 2010	February 15, 2010
	Run 4	February 20, 2010	February 25, 2010
	Run 5	March 2, 2010	March 7, 2010
Seawater	Run 1	March 12, 2010	March 17, 2010
	Run 2	March 22, 2010	March 27, 2010
	Run 3	April 1, 2010	April 6, 2010
	Run 4	April 11, 2010	April 16, 2010
	Run 5	April 21, 2010	April 26, 2010

5. Preparation of test water

For seawater test, salinity was adjusted to >32 PSU by adding artificial seawater powder if necessary. For brackish water test, salinity was adjusted to <22 PSU by adding natural spring water to seawater.

Table 2 Test water quality of G8 criteria

	Salinity		
	>32 PSU	3 – 32 PSU	<3 PSU
Dissolved Organic Carbon (DOC)	>1 mg/l	>5 mg/l	>5 mg/l
Particulate Organic Carbon (POC)	>1 mg/l	>5 mg/l	>5 mg/l
Total Suspended Solids (TSS)	>1 mg/l	>50 mg/l	>50 mg/l

Test water quality was adjusted by adding following materials.

1) For DOC: Potassium hydrogen phthalate 3kg/200m^3 for brackish water.

2) For POC: Shellfish formula feed M-1 (Nosan Corporation) 2.88kg/200m^3 for seawater and 14.4kg/200m^3 for brackish water.

3) For TSS: No adjustment is necessary for both cases.

Addition amount of the above materials were decided by pre-test.

Following planktons were added to satisfy water quality of G8 criteria.

1) Zooplankton: *Brachionus plicatilis*

2) Phytoplankton: *Tetraselmis* sp.

There were enough amounts of heterotrophic bacteria, more than 10^4 cfu/mL, for all the time during the testing in natural seawater.

6. Flow diagram

Flow diagram of land-based testing is shown below.

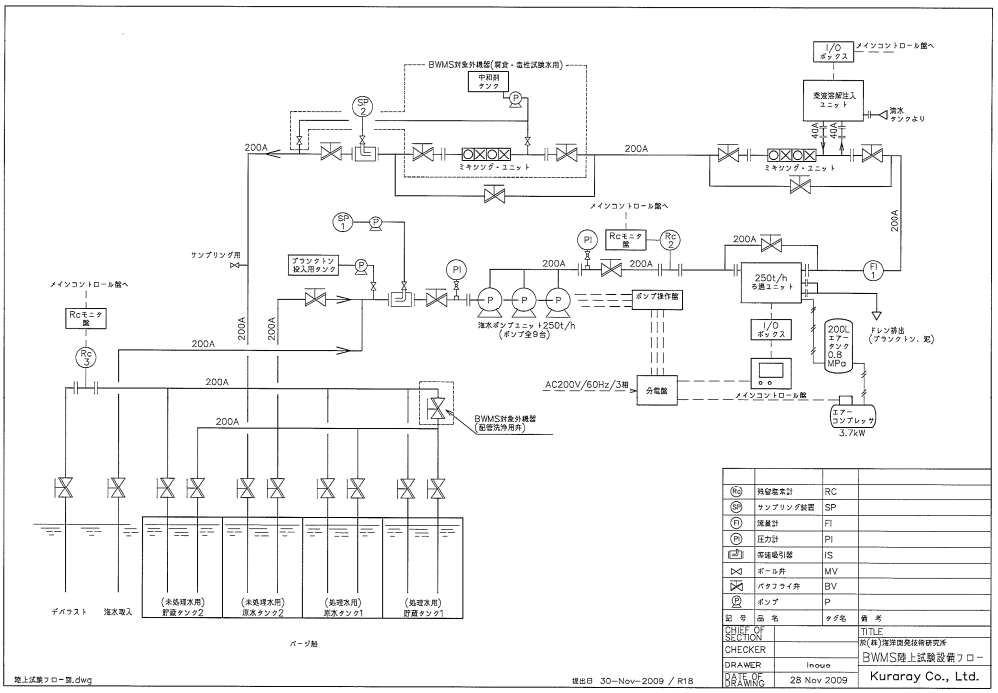

Fig.1 Flow diagram of land-based testing

7. Test procedure

Test cycle is as follows.

1) Preparation of test waters in specified tanks (barge tanks).

2) Ballasting operation and sampling.

3) Storage of ballast water in specified tanks for five days.

4) Deballasting operation and sampling.

In each tank, 200m^3 of test waters, treated waters, and non-treated waters (control waters) were stored/prepared. At deballasting, about 130 m^3 of waters were treated.

Tank capacities were 330 m^3 for treated water and 400 m^3 for control.

8. Test conditions

BWMS model: MICROFADE BWMS MF-250

Treatment Rated Capacity (TRC): 250 m^3/h

Active Substance (AS): Calcium hypochlorite (indicated as TRO)

Maximum dosing of AS: 2 mg/L as TRO

Maximum allowable discharge concentration (MADC) of AS: 0.2 mg/L as TRO

Infusion amount of AS (TRO = 2 mg/L) is adjusted automatically in Main Control Unit (MCU) of MICROFADE BWMS. Flow rate and TRO concentration of prepared AS solution are measured, and injection pump (P2) of AS solution is controlled as follows;

$$P = 2F / C$$

where P: injection pump feed of AS (L/min), F: flow rate (L/min), C: TRO concentration of prepared AS

Standard conditions are P = 2.78 L/min, F = 4,167 L/min (250 m^3/h), and C = 3,000 mg/L.

9. Sampling and analysis

Sampling apparatus and sampling method are described in detail in Quality Assurance Project Plan (QAPP). The sampling apparatus is designed to make “isokinetic flow speed” of both ballasting water and sampling water. The flow rate was 100 L/min.

1) Sampling time and locations

Samples are collected in triplicate (beginning, middle, and end of each operation).

Table 3 Sampling time and locations

	At ballasting (influent)	At deballasting (discharge)
Control water	During ballasting	During deballasting
Treated water	Immediately before BWMS	During deballasting
	Immediately after BWMS	

2) Sampling volume

a) For L-size group (organisms of greater than or equal to 50 micrometer)

More than 1 m^3, samples are collected after concentration by means of sampling apparatus.

b) For S-size group (organisms of greater than or equal to 10 micrometer and less than 50 micrometer)

10 litters for treated water, and 1 litter for control water.

c) For bacteria

3 litters.

d) For water quality analysis

More than five litters for pH, salinity, temperature, and dissolved oxygen (DO).

1 L for POC, 100 mL for DOC, and 2 L for TSS analysis.

3) Analysis method

Analysis method is described in QAPP(including SOP) in detail. The samples are analyzed within 6 hours after sampling or are treated so as to ensure proper analysis.

10. Test results: L-size group (organisms of greater than or equal to 50 micrometer)

For both cases of seawater and brackish water tests, all the test cycles were valid and have met the standard in regulation D-2.

Table 4 Test results of L-size group in seawater (above) and brackish water (below)

Index	Kind of sample water	Require-ments	Results of five test cycles (seawater)				
			1st	2nd	3rd	4th	5th
L-size (ind/m^3)	Influent water for treatment	$\geq 10^5$	4.9×10^5	3.4×10^5	2.4×10^5	2.1×10^5	9.5×10^5
	Control water after 5 days	≥100	7.3×10^4	1.5×10^4	3.0×10^4	1.7×10^4	2.8×10^4
	Treated water after 5 days	<10	< 1	< 1	< 1	< 1	< 1
Phyla/ Divisions	Influent water for treatment	≥ 3	6	7	7	9	7
Species	Influent water for treatment	≥ 5	15	16	17	18	15

Index	Kind of sample water	Require-ments	Results of five test cycles (brackish water)				
			1st	2nd	3rd	4th	5th
L-size (ind/m^3)	Influent water for treatment	$\geq 10^5$	1.0×10^5	4.0×10^5	6.5×10^5	2.0×10^5	4.4×10^5
	Control water after 5 days	≥100	3.4×10^2	7.8×10^3	4.5×10^4	6.3×10^3	1.9×10^4
	Treated water after 5 days	<10	5.3	< 1	< 1	< 1	< 1
Phyla/ Divisions	Influent water for treatment	≥ 3	4	4	3	3	3
Species	Influent water for treatment	≥ 5	13	12	9	11	9

Results are the average of triplicate samples in each test cycle.
Numbers of phyla / divisions and species are shown by counting all the observed organisms in the triplicate samples.

11. Test results: S-size group (organisms of greater than or equal to 10 micrometer and less than 50 micrometer)

For both cases of seawater and brackish water tests, all the test cycles were valid and have met the standard in regulation D-2.

Table 5 Test results of S-size group in seawater (above) and brackish water (below)

Index	Kind of sample water	Require-ments	Results of five test cycles (seawater)				
			1st	2nd	3rd	4th	5th
S-size (ind/mL)	Influent water for treatment	$\geq 10^3$	3.3×10^3	2.1×10^3	2.8×10^3	2.5×10^3	2.5×10^3
	Control water after 5 days	≥100	1.4×10^2	2.0×10^2	1.9×10^2	1.4×10^2	6.5×10^2
	Treated water after 5 days	<10	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Phyla/ Divisions	Influent water for treatment	≥ 3	4	4	4	4	4
Species	Influent water for treatment	≥ 5	12	27	24	29	25

Index	Kind of sample water	Require-ments	Results of five test cycles (brackish water)				
			1st	2nd	3rd	4th	5th
S-size (ind/mL)	Influent water for treatment	$\geq 10^3$	1.2×10^3	1.7×10^3	2.4×10^3	3.7×10^3	1.4×10^3
	Control water after 5 days	≥100	1.5×10^2	3.2×10^2	1.7×10^2	1.4×10^2	2.7×10^2
	Treated water after 5 days	<10	0.76	< 0.01	0.03	< 0.01	0.08
Phyla/ Divisions	Influent water for treatment	≥ 3	4	4	3	4	4
Species	Influent water for treatment	≥ 5	14	9	6	6	11

Results are the average of triplicate samples in each test cycle.
Numbers of phyla / divisions and species are shown by counting all the observed organisms in the triplicate samples.

12. Test results: Bacteria

Heterotrophic bacteria, *Escherichia coli*, intestinal *Enterococci*, and toxicogenic *Vibrio cholerae* (serotypes O1 and O139) were analyzed in sample water.

For both cases of seawater and brackish water tests, all the test cycles were valid and have met the standard in regulation D-2.

Table 6 Test results of bacteria in seawater (above) and brackish water (below)

Index	Kind of sample water	Require-ments	Results of five test cycles (seawater)				
			1st	2nd	3rd	4th	5th
Heterotrophic bacteria (cfu/mL)	Influent water for treatment	$\geq 10^4$	4.2×10^4	5.5×10^5	4.7×10^4	2.6×10^5	4.6×10^5
E. coli (cfu/100mL)	Influent water for treatment	–	ND	ND	ND	ND	ND
	Control water after 5 days	-	ND	ND	ND	ND	ND
	Treated water after 5 days	<250	ND	ND	ND	ND	ND
Intestinal *Enterococci* (cfu/100mL)	Influent water for treatment	–	ND	ND	0.55	4.9×10^3	ND
	Control water after 5 days	-	ND	ND	25	1.3×10^2	4.8×10^3
	Treated water after 5 days	<100	ND	ND	ND	ND	ND
Vibrio cholerea (cfu/100mL)	Influent water for treatment	–	ND	ND	ND	ND	ND
	Control water after 5 days	-	ND	ND	ND	ND	ND
	Treated water after 5 days	<1	ND	ND	ND	ND	ND

Index	Kind of sample water	Require-ments	Results of five test cycles (brackish water)				
			1st	2nd	3rd	4th	5th
Heterotrophic bacteria (cfu/mL)	Influent water for treatment	$\geq 10^4$	7.2×10^5	9.0×10^5	6.2×10^5	7.3×10^5	4.9×10^5
E. coli (cfu/100mL)	Influent water for treatment	–	ND	ND	ND	ND	ND
	Control water after 5 days	-	ND	ND	ND	ND	ND
	Treated water after 5 days	<250	ND	ND	ND	ND	ND
Intestinal *Enterococci* (cfu/100mL)	Influent water for treatment	–	2.4×10^2	2.1×10^1	ND	ND	ND
	Control water after 5 days	-	1.2	3.8×10^1	ND	ND	9.0
	Treated water after 5 days	<100	ND	0.35	2.3	0.21	ND
Vibrio cholerea (cfu/100mL)	Influent water for treatment	–	ND	ND	ND	ND	ND
	Control water after 5 days	-	ND	ND	ND	ND	ND
	Treated water after 5 days	<1	ND	ND	ND	ND	ND

Results are the average of triplicate samples in each test cycle. ND means bacteria were not detected. *Vibrio cholera* are toxicogenic *Vibrio cholera* (serotypes O1 and O139).

13. Test results: Water quality

Particulate organic carbon (POC), dissolved organic carbon (DOC), and total suspended solid (TSS) in test water were analyzed as well as pH, salinity, temperature, and dissolved oxygen (DO).

For both cases of seawater and brackish water tests, POC, DOC, and TSS requirements were satisfied in influent water. For salinity, all seawater samples showed more than 32 PSU and all brackish water samples showed in the range of 3 to 22 PSU. Salinity ranges of both test cases were separated by at least 10 PSU.

Table 7 Test results of water quality in seawater (above) and brackish water (below)

Index	Kind of sample water	Require-ments	Results of five test cycles (seawater)				
			1st	2nd	3rd	4th	5th
Salinity (PSU)	Influent water for treatment	> 32	32.6	32.6	33.0	33.1	33.2
DOC (mg/L)	Influent water for treatment	≥ 1	2.8	1.1	1.0	1.1	1.1
POC (mg/L)	Influent water for treatment	≥ 1	4.4	5.5	3.7	6.2	8.2
TSS (mg/L)	Influent water for treatment	≥ 1	15	9.4	13	16	18
pH	Influent water for treatment	-	8.2	8.3	8.4	8.4	8.4
Temperature (°C)	Influent water for treatment	-	12.7	12.7	14.4	15.7	15.3
DO (mg/L)	Influent water for treatment	-	8.0	8.0	7.2	7.4	7.3
Turbidity (NTU)	Influent water for treatment	-	87	53	50	46	36

Index	Kind of sample water	Require-ments	Results of five test cycles (brackish water)				
			1st	2nd	3rd	4th	5th
Salinity (PSU)	Influent water for treatment	3 – 22	20.7	18.7	20.6	19.9	20.2
DOC (mg/L)	Influent water for treatment	≥ 5	8.5	6.5	7.0	18.7	13.7
POC (mg/L)	Influent water for treatment	≥ 5	8.6	5.1	19	51	53
TSS (mg/L)	Influent water for treatment	≥ 50	94	59	67	184	137
pH	Influent water for treatment	-	8.1	8.0	8.1	8.1	8.3
Temperature (°C)	Influent water for treatment	-	10.9	11.3	9.3	11.0	14.3
DO (mg/L)	Influent water for treatment	-	8.8	9.3	9.4	6.4	8.9
Turbidity (NTU)	Influent water for treatment	-	226	118	176	329	230

Results are the average of triplicate samples in each test cycle.

14. Test results: Operation conditions

During the land-based testing, all the operation conditions were recorded and evaluated that everything was normal and appropriate.

Detailed conditions were as follows;

Ballast flow rate:	250 to 268 m^3/h
TRO concentration at RC1:	3000 ± 290 mg/L
TRO concentration at dosage (calculation):	2.00 ± 0.03 mg/L
TRO concentration in treated ballast water (actual):	
	1.79 to 1.97 mg/L (seawater tests)
	0.44 to 1.83 mg/L (brackish water tests)
TRO concentration at discharge (actual):	0.00 to 0.08 mg/L (seawater tests)
	0.00 to 0.09 mg/L (brackish water tests)